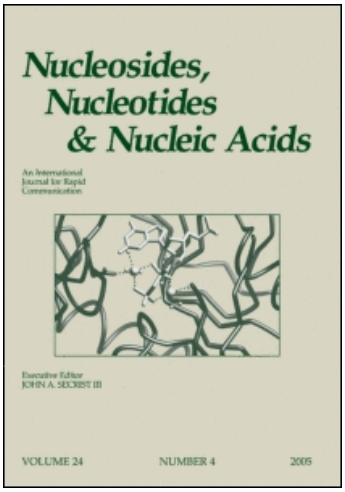

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SYNTHESIS OF ACETYLATED α- AND β-L-FUCOSYL ESTERS OF NUCLEOSIDE 5'-MONOPHOSPHATES BY THE ORTHOESTER ROUTE

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Abstract: Reaction of 3,4-di-O-acetyl-1,2-O(ethyl orthoacetyl)-α-L-fucopyranose with UMP and dTMP in N,N-dimethyl formamide gave the corresponding nucleoside-5'-(2,3,4-tri-O-acetyl-L-fucopyranosyl phosphoric acids) as a mixture of the α- and β-anomers.

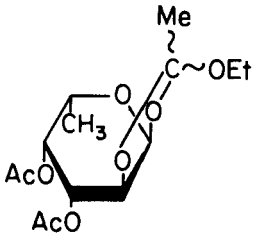
Glycosyl esters of nucleotides are essential intermediates in glycosyl-transfer reactions that lead to the biosynthesis[1] of complex carbohydrates, glycoproteins, proteoglycans, and glycolipids. In a previous report, we demonstrated[2] that the addition of nucleoside 5'-monophosphoric acids to a D-glucose orthoester gave exclusively the β-anomer of the D-glucosyl ester of the nucleotide, the expected product of addition to a sugar orthoester. By contrast, we found that in a corresponding synthesis involving addition of a nucleoside 5'-diphosphoric acid to a D-glucose orthoester, the α-anomer was the exclusive product.[3] It is obviously important to be able to control the anomeric configuration in these syntheses, and to that end, we have chosen to study the L-fucose series, as fucose derivatives undergo the transformation especially readily[4]. We now report

that the reaction in the L-fucose series gives rise to a mixture of the α - and β -L-fucosyl esters of the nucleotide.

RESULTS AND DISCUSSION

A solution of 3,4-di-O-acetyl-1,2-O-(1-ethoxyethylidene) -α -L-fucopyranose (1) and anhydrous thymidine-5'-monophosphoric acid (dTMP) in dry N,N-dimethylformamide (DMF) showed a single, new, uv-absorbing spot, R_F 0.45 (t.l.c., solvent B), after 20 h at 25°. A new phosphate-containing spot also appeared upon electrophoresis at R picrate (R_p) 0.58, corresponding to the expected thymidine monophosphate fucosyl ester, whereas dTMP has R_p 1.37. The material having R_p 0.58 was isolated by preparative, thin-layer chromatography. Elemental analysis suggested that this material was the tri-O-acetyl-L-fucopyranosyl ester of thymidine 5'-monophosphate (2).

The ^{13}C-nmr spectrum clearly showed a mixture of the anomers. As expected, the resonance of the L-fucosylα-anomeric carbon atom (C-1",α) appeared at 93.2 ppm, and was split into a doublet by the phosphorus nucleus, $J_{P-C_{1"}}$ 4.8 Hz. Similarly, the C-1" resonance appeared at 96.3 ppm, and was also split into a doublet by the phosphorus nucleus. The C-5' and C-2" resonances for the α-anomer were split into two doublets, having $J_{P-C5'}$ 3.6 and $J_{P-C2"}$ 7.2 Hz. The ^{31}P-nmr spectrum confirmed the presence of α -and β -anomers, as, in the proton-decoupled spectrum, one phosphorus resonance (β) appeared at -1.95 ppm and the other phosphorus resonance (α) appeared at -1.98 ppm. This difference (0.03 ppm) in chemical shift is consistent with that observed by Rosner et al[5]. The proton-coupled ^{31}P-nmr spectrum showed a complex pattern of two sextuplets. The 300-MHz, ^{1}H-nmr spectrum of 2 showed the H-1" (α) signal as a doublet of doublets, at δ 5.54, which collapsed to a doublet, $J_{1,2}$ 4.4Hz, on decoupling phosphorus. The H-1" (β) signal was



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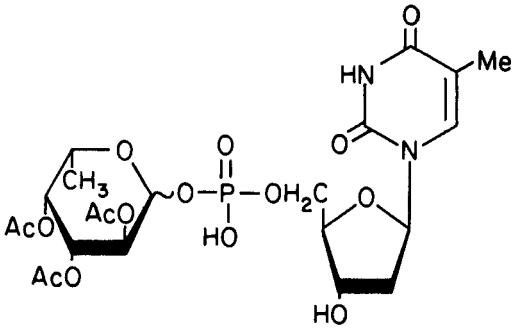

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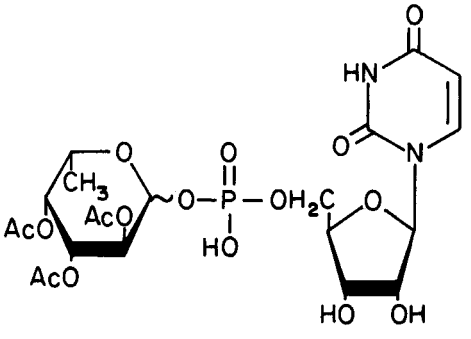

3

buried under the HOD peak. The i.r. spectrum contained strong bands at 1760 (C=0) and 1250 cm^{-1} (P=0).

Compound **2** was subjected to analysis by enzymic means; 0.5 μ mol was treated with alkaline phosphomonoesterase and with phosphodiesterase. The phosphodiesterase cleaved **2** to dTMP, whereas the phosphomonoesterase

was without effect. In contrast, control experiments showed that the phosphodiesterase had no effect on dTMP, but that the phosphomonoesterase catalyzed the hydrolysis of dTMP to thymidine. Fucose, dTMP, and thymine were formed when **2** was treated with 0.3M HCl for 96 h at 25°.

Compound **3** was prepared by an analogous procedure and showed hydrolytic behavior analogous to that described for **2**. Nmr data for **3** are given in Table 1.

The specific rotations of **2** and **3** suggest that both consist of 60% of the α- and 40% of the β-anomer, on the assumption of additivity of the rotations of the nucleotide [2] and acetylated sugar moieties[6].

The product resulting from addition to a sugar orthoester normally has the 1,2-trans configuration, although numerous cases of cis-

TABLE 1

^{13}C- and ^{31}P- Chemical Shifts (p.p.m.) of L-Fucosyl Esters of Nucleotides

Compound		2	4	5	6	5-Me	1'	2'	3'
2	α	152.2	166.8	112.2	138.1	12.2	85.5	39.3	71.9
	β	152.2	166.8	112.0	137.8	12.2	85.5	39.3	71.9
3	α	152.5	166.6	103.4	142.5		89.4	74.3	70.7
	β	152.5	166.6	103.4	142.2		89.4	74.3	70.7

Compound		4'	5'	1"	2"	3"	4"	5"	6"	P
2	α	86.0[a]	65.9d	93.2d	68.4d	68.9	71.6	66.8	15.5	-1.98
	β	86.0[a]	65.9d	96.3d	71.3[a]	71.8	70.4	71.6	15.5	-1.95
3	α	83.6d	65.7d	93.3d	68.7d	69.1	71.5	66.9	15.7	-1.99
	β	83.6d	65.7d	96.4d	71.5[a]	72.1	70.4	71.5	15.7	-1.90

Coupling constants (Hz)

Compound		$J_{P, C1"}$	$J_{P, C4'}$	$J_{P, C5'}$	$J_{P, C2"}$
2	α	4.8	[a]	3.6[a]	7.2[a]
	β	~5	[a]		
3	α	4.8	8.4[a]	4.8[a]	7.2[a]
	β	~5			

[a] Splitting obscured by overlapping resonances.

products have been observed[7]. The mechanism of the anomerization reactions which appear to take place here is not yet understood. The formation of the α-anomer for the reaction of a D-glucose orthoester with a nucleoside diphosphoric acid is not simply due to the longer time required for the reaction. We have allowed reactions of a nucleoside monophosphoric acid with the D-glucose orthoester to proceed for 8-20 days as in the reaction of the nucleoside diphosphoric acid, but there was still no trace of the α-anomer. Anomerization during synthesis of sugar phosphates by several routes has been observed[8,9]. These processes may be analogous to the better known anomerizations of glycosyl halides and acetates[10].

EXPERIMENTAL

General methods---These were the same as those described previously[2]. Solvent A was 3:1 ether--light petroleum; and solvent B, 60:35:6 chloroform--methanol--water.

Preparations contained some $CaSO_4$ derived from the tlc plates. This proved difficult to remove. That the compounds are otherwise analytically clean is shown not only by the elemental analyses but also by the extinction coefficients which agree with literature values when corrected for the reported quantity of $CaSO_4$.

Thymidine 5'(2,3,4-tri-O-acetyl-L-fucopyranosyl phosphoric acid)--**2**--To a solution of 3,4-di-O-acetyl-1,2-O-(1-ethoxyethylidene)-α-L-fucopyranose (1; 0.36g, 1.13 mmol), as a mixture of the exo- and endo-isomers and the glycoside[11], in dry[12] DMF (2 mL) at room temperature was added anhydrous thymidine-5'-phosphoric acid (0.134 g, 0.42 mmol) prepared by treatment of the sodium salt with Dowex -50 resin, and the mixture was stirred for 20 h at room temperature. T.l.c. (solvent A) then showed diminution of the orthoester spot (R_F 0.68) and concurrent appearance of material having zero mobility. T.l.c. (solvent B) showed the appearance of a new spot, at R_F 0.45. Electrophoresis at pH 7.9 showed two phosphorus-containing spots,

one corresponding to dTMP (mobility relative to picrate ion, R_p 1.37) and the other to **2** (R_p 0.58); the latter was isolated by preparative, thin-layer chromatography on plates of silica gel with solvent B; yield 0.06g (24.3%). $[\alpha]_D^{25}$ -64° (c 0.6, water). $\lambda_{max}^{pH\ 6.3}$ 267 nm (ϵ mM 9.43); lit.[13] for thymine, ϵ mM 9.65.

Anal.:Calc. for $C_{22}H_{31}N_2O_{15}P,1.4CaSO_4, H_2O$:C,32.9; H,4.11; N,3.49;P,3.86. Found:C,32.6; H,4.77;N,3.6; P,4.17. Atomic ratios, C/N/P: 22/2.08/1.09.

Uridine 5'-(2,3,4-tri-O-acetyl-L-fucopyranosyl phosphoric acid) (3).---Uridine-5'-phosphoric acid, like thymidine-5'-phosphoric acid, readily dissolves in DMF. The synthesis was analogous to that of **2**. Compound **3** (R_F 0.5, solvent B; R_p 0.64) was isolated in a yield of 0.06g (25%);$[\alpha]_D^{25}$ -64.9° (c 0.55, water); $\lambda_{max}^{pH\ 6.3}$ 262 nm (ϵ mM 10.00); lit.[13] for UMP ϵ 10.0. The ir spectrum showed C=O and P=O absorption bands at 1760 , and 1250 cm^{-1}, respectively.

Anal.: Calc. for $C_{21}H_{29}N_2O_{16}P,3CaSO_4,H_2O$: C,24.23; H,3.17;N,2.69;P,2.98. Found:24.43;H,3.76;N,2.54; P,3.27. Atomic ratios, C/N/P: 21/1.86/1.08.

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REFERENCES

1. H. Nikaido and W.Z. Hassid, Adv. Carbohydr. Chem. Biochem., 26, 351-483,(1971) .
2. M.A. Salam and E.J. Behrman, Carbohydr. Res., 102, 139-146 (1982) .

3. M.A. Salam and E.J. Behrman, Carbohydr. Res., 101, 339-342 (1982).

4. H.S. Prihar, J.-H. Tsai, S.R. Wanamaker, S.J. Duber, and E.J. Behrman, Carbohydr. Res., 56, 315-324 (1977).

5. M.R. Rosner, H.G. Khorana and A.C. Satterthwait, J. Biol. Chem., 254, 5918-5925 (1979).

6. F.J. Bates and Associates, Polarimetry, Saccharimetry, and the Sugars, Natl. Bur. Stand. (U.S.) Circ., 440, 716 (1942).

7. N.K. Kochetkov and A.F. Bochkov, Recent Dev. Chem. Nat. Carbon Compd., 4, 75-191, (1971); P.J. Garegg and I. Kvarnström, Acta Chem. Scand., Ser. B30, 655-658 (1976).

8. P.A. Hedba, E.J. Behrman, and G.A. Barber, Arch. Biochem. Biophys., 194, 496-502 (1979).

9. D.L. MacDonald, Carbohydr. Res., 3, 117-120 (1966); R.R. Schmidt, M. Stumpp, and J. Michel, Tetrahedron Lett., 23, 405-408 (1982).

10. B. Capon, Chem. Rev., 69, 407-498 (1969).

11. J.H. Tsai and E.J. Behrman, Carbohydr. Res., 64, 297-301, (1978).

12. M.A. Salam and E.J. Behrman, Carbohydr. Res., 90, 83-89, (1981).

13. G.D. Fasman (Ed.), Handbook of Biochemistry and Molecular Biology, 3rd. edition., Nucleic Acids, Vol. 1, CRC Press, Cleveland, Ohio 1975.

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